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Extraction of high-quality genomic DNA from latex-containing plants☆

An Michiels,[a,*] Wim Van den Ende,[a] Mark Tucker,[b] Liesbet Van Riet,[a] and André Van Laere[a]

[a] *Laboratory of Plant Development, Institute of Botany and Microbiology, K. U. Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium*
[b] *USDA/ARS/SGIL, Building 6, BARC-West, 10300, Baltimore Ave., Beltsville, MD 20705-2350, USA*

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Abstract

The isolation of intact, high-molecular-mass genomic DNA is essential for many molecular biology applications including long PCR, endonuclease restriction digestion, Southern blot analysis, and genomic library construction. Many protocols are available for the extraction of DNA from plant material. However, for latex-containing Asteraceae (Cichorioideae) species, standard protocols and commercially available kits do not produce efficient yields of high-quality amplifiable DNA. A cetyltrimethylammonium bromide protocol has been optimized for isolation of genomic DNA from latex-containing plants. Key steps in the modified protocol are the use of etiolated leaf tissue for extraction and an overnight 25 °C isopropanol precipitation step. The purified DNA has excellent spectral qualities, is efficiently digested by restriction endonucleases, and is suitable for long-fragment PCR amplification.

Keywords: DNA extraction; CTAB; Cichorioideae; Latex; Long PCR

Cichorioideae plants belong to the Asteraceae family and produce bitter latex on the surface of wounded leaves and roots. The major components of latex were shown to be conjugates of guaianolide sesquiterpene lactone and lactucin, which are produced constitutively as secondary metabolites and as phytoalexins [1]. In addition to these polyphenolic conjugates, Cichorioideae species often contain high concentrations of fructan oligo- and polysaccharides, which can make up to 80% of the root dry weight [2]. The presence of polyphenolics together with the high polysaccharide content makes the isolation of high-quality intact nucleic acids problematic. In addition to complicating extraction residual polyphenolics and polysaccharides interfere in enzymatic reactions such as PCR and endonuclease restriction digestion [3].

Nevertheless, several successful DNA extraction methods for plant species containing polyphenolic compounds and polysaccharides have been developed [4–10]. However, published procedures tested were inadequate for the extraction of high-quality DNA from latex-containing Cichorioideae plants. Moreover, recent publications demonstrate the use of hydrolytic enzymes [6] or ion-exchange resins [7,8] to remove polysaccharides from nucleic acid solutions. In a more commonly used procedure, polysaccharide coprecipitation is avoided by adding a selective precipitant of nucleic acids, i.e., cetyltrimethylammonium bromide (CTAB)[1] to keep polysaccharides in solution [11].

CTAB DNA purification methods extract high quantities of pure DNA from a variety of different plant tissues, e.g., cotton, blackcurrant, ferns, fruit trees, and conifers [12–14]. However, none of these protocols is suitable for extracting high-quality DNA from latex-containing plants. In this report, we describe an

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* Corresponding author. Fax: +32-16-321967.
E-mail address: an.michiels@bio.kuleuven.ac.be (A. Michiels).

[1] *Abbreviations used:* PVP, polyvinylpyrrolidone; CTAB, cetyltrimethylammonium bromide; 1-FEH, fructan 1-exohydrolase.

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optimized CTAB protocol that consistently yields large amounts of high-quality amplifiable DNA with as little as 1 g fresh weight starting material.

Material and methods

Plant material

DNA was isolated from the latex-containing plants *Cichorium intybus* var. *sativum*, *C. intybus* var. *foliosum*, *Taraxacum officinale*, and *Lactuca sativa*. Seeds were germinated in pots and maintained in the greenhouse during the summer. Etiolated leaves were obtained by harvesting roots before flowering (from 2 to 4 months old) and replanting in dark growth chambers at 20 °C. Four weeks later, etiolated leaves were collected.

Preparation of buffers

The extraction buffer used for the initial homogenization contained 100 mM Tris, pH 8, 1.4 M NaCl, 20 mM EDTA, pH 8, and 0.2% (v/v) β-mercaptoethanol. The extraction buffer was autoclaved and 2% polyvinylpyrrolidone (PVP; Sigma, St. Louis, MO; MW 10,000) and 2% CTAB were added immediately before use. The wash solution contained 10 mM ammonium acetate and 70% ethanol. TE buffer contained 10 mM Tris, pH 8, and 1 mM EDTA.

DNA extraction methods

A CTAB DNA isolation procedure [11] was used for comparison to the optimized protocol detailed in Table 1. In addition, an anion exchange chromatography-based DNeasy Plant Mini Kit was used as a commercially available kit (Qiagen, Valencia, CA, USA).

PCR amplification

Specific fructan 1-exohydrolase (1-FEH) primers were designed to amplify *Cichorium* genomic DNA [15,16]. (F_1: 5′TAAAGTCATCGATCCCCAAACACACA3′; R_1: 5′AACGTTGCTGCATACGGATTGTATTG3′; F_2: 5′CCAGGGAGTGTACCATTTCTTCTACCA3′; R_2: 5′CAACTGTTTCCCATTTCTGTCGATCC3′; F_3: 5′CCAATACAACCCTTACGGTCCACTCT3′; R_3: 5′GCCATTGTACCAACTGTTTCCGGTTA3′; F_4: 5′TCGTTTCCTAGGAGCATCTGGCTTAG3′; R_4: 5′AGGAACTATTTGTGCCCTCTTCATGC3′). Reaction mixtures (15 µl) contained 20 ng template DNA, 0.6 U *Taq* polymerase (Gibco-BRL, Gaitherburg, MD, USA), 1× PCR buffer, 1.1 mM $MgSO_4$, 0.2 mM dNTP, 0.4 µM of each Forward (F) and Reverse (R) primer. The PCR mixture was subjected to the following PCR program: 1 cycle 94 °C for 5 min, 5 cycles 95 °C for 30 s, 67 °C for 4 min, 30 cycles 95 °C for 35 s, 65 °C for 4 min, with final extension 72 °C for 7 min. The samples were analyzed by agarose gel electrophoresis.

Endonuclease digestion

C. intybus genomic DNA (5 µg) was incubated with 10 units each of *Eco*RI, *Eco*RV, *Sma*I, *Xba*I, and *Xho*I (Boehringer Mannheim, Mannheim, Germany) in the recommended buffer at 37 °C for 4 h. DNA digestion was assayed by visual inspection after agarose gel electrophoresis.

Results and discussion

Genomic DNA amplifications, Southern blot analysis, and library construction necessitate the successful isolation of high-quality DNA. Previous failure to isolate large-molecular-weight DNA of sufficient purity

Table 1
Modified CTAB extraction protocol for latex-containing plants

1. Grind etiolated fresh leaves (1 g) to a powder in liquid nitrogen in a chilled mortar. Scrape the powder directly into 15 ml of 60 °C preheated extraction buffer and mix carefully.
2. Incubate the sample at 60 °C for 60 min with occasional mixing to avoid aggregation of the homogenate.
3. Add 15 ml chloroform:isoamylalcohol (24/1) to the extract and vortex thoroughly. Centrifuge for 5 min at 2500*g* (20 °C). Transfer the upper phase to a clean tube. Repeat the chloroform:isoamylalcohol extraction twice to clear the aqueous phase.
4. Mix the aqueous phase with 2/3 volume of isopropanol by inversion and incubate at 25 °C overnight to precipitate the nucleic acids. It is important to use 25 °C rather than the more commonly used 4 °C.
5. Centrifuge at 5000*g* for 10 min (20 °C). Gently pour off the supernatant and add 15 ml wash solution.
6. Rinse the pellet with wash solution and incubate at room temperature for at least 15 min. Centrifuge at 2500*g* for 6 min (20 °C). Repeat the washing step.
7. Pour off supernatant and allow the pellet to dry briefly in air. Resuspend in 1 ml TE buffer and incubate at 37 °C with RNase A to a final concentration of 10 µg/ml for 30 min.
8. Add one volume of phenol and mix vigorously to form an emulsion. Centrifuge for 5 min at 2500*g* (20 °C). Transfer the upper phase to a sterile microcentrifuge tube and repeat the extraction with phenol:chloroform:isoamylalcohol (25/24/1) and with chloroform: isoamylalcohol (24/1).
9. Add 7.5 M NH_4Ac, pH 7.7, to a final concentration of 2.5 M and 2 volumes of cold ethanol to the transferred aqueous phase, mix, and incubate on ice for a few minutes. Precipitate by centrifuging at 5000*g* for 10 min at 4 °C.
10. Rinse the pellet twice with 70% ethanol, air dry, and resuspend in 30 µl DNase-free water.

from latex-containing plants could be attributed to the presence of polysaccharides and polyphenolic latex compounds. To overcome this problem, we optimized the precipitation temperature and duration. In addition, the influence of the age and growth conditions of the plant material was tested.

In general, the quantity and quality of isolated DNA depends on precipitation temperature and duration (Table 2). Low-temperature precipitation increased DNA yield from etiolated *Cichorium* leaves but spectrophotometric analysis demonstrated a clear reduction of DNA purity. Isopropanol precipitation at −20 °C overnight yields 203 μg DNA per 1 g of leaf tissue. Absorbance ratios at A_{260}/A_{230} were approximately 0.53, indicating large amounts of impurities caused by coprecipitation of peptides, aromatic compounds, and polysaccharide and/or phenolic complexes (Table 2). Precipitation overnight at 25 °C resulted in a reduction of total DNA extraction yield (140 μg/g tissue) but spectrophotometric analysis at A_{260}/A_{230} revealed a 2.14 ratio. Warm-temperature precipitation indeed led to a clear reduction of contaminating components during precipitation.

DNA yields from etiolated *Cichorium* leaves using the modified CTAB method ranged from 120 to 160 μg/g fresh tissue with A_{260}/A_{280} close to 2.00 and A_{230}/A_{260} around 2.14, indicating very little contamination of the DNA fraction by proteins, polysaccharides, and aromatic compounds. Extraction of green leaves yielded smaller amounts of poor-quality DNA (Table 3). Etiolated leaves appear to be excellent tissue for the isolation of genomic DNA. These results are consistent with the results of Dabo et al. [17] who observed major difficulties when a DNA extraction method from etiolated cotton cotyledons was applied to green cotyledons. They concluded that photosynthetic active tissue contains phenolic compounds that oxidize during extraction and irreversibly interact with proteins and nucleic acids to form a gelatinous matrix. This matrix might inhibit proper extraction, amplification, and digestion of DNA.

PVP forms complex hydrogen bonds with latex lactones, lactucin, and other phenolics and coprecipitates with cell debris upon lysis. When the extract is centrifuged in the presence of chloroform, the PVPcomplexes accumulate at the interface between the organic and the aqueous phases [14,18,19]. CTAB binds to fructans and other polysaccharides and forms complexes that are removed during subsequent chloroform extraction [20]. Polysaccharides interfere with biological enzymes such as polymerases, restriction endonucleases, and ligases [21]. PVP and CTAB were included only as precautionary measures and were not specifically evaluated. *Cichorium* DNA endonuclease digestion with five different endonucleases (*Eco*RI, *Eco*RV, *Sma*I, *Xba*I, and *Xho*I) showed complete digestion using the modified protocol. DNA extracted by the standard CTAB

Table 2
DNA quantity and purity using the modified CTAB protocol to isolate DNA from etiolated leaves of *Cichorium intybus* var. *foliosum*, including different isopropanol incubation temperatures and precipitation times

Incubation time	Precipitation temperature	μg DNA[a]	A_{260}/A_{280}	A_{260}/A_{230}
3 h	4 °C	78 ± 3	1.85 ± 0.09	1.53 ± 0.15
3 h	25 °C	49 ± 5	1.77 ± 0.05	1.68 ± 0.21
Overnight	−20 °C	203 ± 21	1.93 ± 0.10	0.53 ± 0.24
Overnight	4 °C	182 ± 23	1.87 ± 0.13	1.03 ± 0.11
Overnight	14 °C	144 ± 14	1.93 ± 0.08	1.75 ± 0.03
Overnight	25 °C	140 ± 13	1.96 ± 0.03	2.14 ± 0.04

Data are means ± SE ($n = 3$).
[a] For 1 g of plant tissue.

Table 3
DNA quantity and purity using the standard CTAB method [4], a commercially available extraction kit, and the modified CTAB protocol to isolate DNA from *Cichorium intybus* var. *foliosum*

Method	Tissue	μg DNA[a]	A_{260}/A_{280}	A_{260}/A_{230}
Commercial kit	Leaves from 15-day-old plants	40 ± 6	1.55 ± 0.06	0.89 ± 0.08
	Leaves from 30-day-old plants	24 ± 6	1.58 ± 0.09	0.73 ± 0.10
	Etiolated leaves	53 ± 8	1.77 ± 0.06	0.93 ± 0.03
CTAB	Leaves from 15-day-old plants	45 ± 7	1.68 ± 0.12	1.23 ± 0.14
	Leaves from 30-day-old plants	50 ± 12	1.47 ± 0.05	1.05 ± 0.07
	Etiolated leaves	78 ± 3	1.85 ± 0.09	1.53 ± 0.15
Optimized CTAB	Leaves from 15-day-old plants	47 ± 9	1.87 ± 0.04	1.49 ± 0.07
	Leaves from 30-day-old plants	43 ± 4	1.82 ± 0.10	1.38 ± 0.08
	Etiolated leaves	140 ± 13	1.96 ± 0.03	2.14 ± 0.04

Leaves from 15 or 30 days old plants or etiolated leaves were extracted. Data are means ± SE ($n = 4$).
[a] For 1 g of plant tissue.

method appeared to be unsuitable for endonuclease restriction digestion (Fig. 1).

Similar results were obtained for PCR amplification analysis. Standard extraction methods resulted in DNA that is difficult to amplify, especially fragments larger than 1 kb, while the DNA isolated by the modified isolation technique is amplifiable by PCR (Fig. 2). Genomic DNA was successfully isolated from *C. intybus* var. *sativum*, *C. intybus* var. *foliosum*, *T. officinale*, and *L. sativa* (Fig. 3). The average length of the isolated DNA was more than 30 kb. The extracted DNA from all four species was suitable for digestion and amplification, demonstrating the purity of the samples (results not shown).

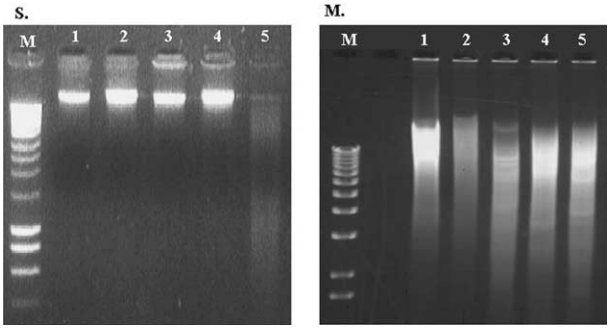

Fig. 1. Endonuclease restriction digest of genomic DNA from *Cichorium intybus* var. *foliosum* etiolated leaves extracted by the standard (S.) and the modified (M.) CTAB protocol. Endonuclease enzymes *Xba*I (lane 1), *Xho*I (lane 2), *Sma*I (lane 3), *Eco*RV (lane 4), and *Eco*RI (lane 5) were used. M, Invitrogen 1-kb molecular weight marker.

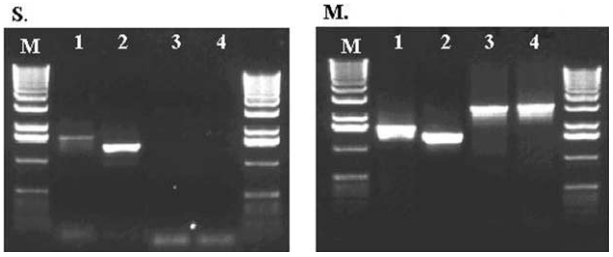

Fig. 2. PCR amplification of genomic DNA from *Cichorium intybus* var. *foliosum* etiolated leaves extracted by standard (S.) and modified CTAB (M.) protocols. Primer combination R_1–F_1 (lane 1), R_2–F_2 (lane 2), R_3–F_3 (lane 3), and R_4–F_4 (lane 4). M, Invitrogen 1-kb molecular weight marker.

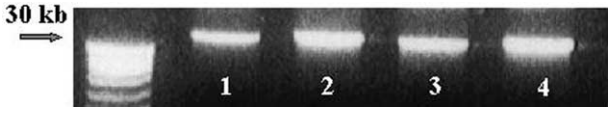

Fig. 3. Genomic DNA extracted by the modified CTAB method from etiolated leaves of *Cichorium intybus* var. *sativum* (lane 1), *C. intybus* var. *foliosum* (lane 2), *Taraxacum officinale* (lane 3), and *Lactuca sativa* (lane 4).

In summary, the age and growth conditions of the plant material influence the isolation efficiency of high-quality DNA. The extraction of etiolated leaf tissue from latex-containing species results in increased yields of high-quality DNA. The Doyle and Doyle [11] protocol is successfully optimized by including an overnight isopropanol precipitation step at 25 °C and by adding PVP to the extraction buffer. Although the optimization slightly reduces the quantity of the isolated DNA, the quality increase actually permits further genomic analyses.

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